*Kidney International, Vol. 64 (2003), pp. 2150–2154*

# Urine calcium and volume predict coverage of renal papilla by Randall's plaque

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**Urine calcium and volume predict coverage of renal papilla by Randall's plaque.**

*Background*. Renal papillary plaques are common in calcium stone formers. We hypothesized that plaque should increase directly with urine calcium excretion, and inversely with urine volume. To test this, we measured papillary plaque areas in both idiopathic calcium oxalate stone formers and nonstone formers and examined 24-hour urine data to identify significant correlations.

*Methods*. Fourteen stone formers and four nonstone forming controls underwent papillary mapping with flexible nephroscopy. For each papillum, representative still images and moving pictures expert group (MPEG) movies were used to identify plaque extent and papillary borders. The mean fractional plaque coverage for each polar region (upper, inter, lower) and per papillum was calculated. The relationship of the plaque coverage data to urine measurements was assessed with general multivariate linear modeling.

*Results*. Mean polar fractional plaque coverage was higher in the calcium oxalate stone formers (7.4% vs. 0.5%, $P = 0.012$) as was mean fractional plaque per papillum (7.6% vs. 0.6%, $P = 0.011$). When correlating mean polar plaque coverage to urine data, urine volume and calcium excretion were the only measurements with independent relationships to plaque ($P = 0.002$, adjusted multiple $R^2 = 0.521$), with higher calcium and lower volume increasing coverage. The same relationships hold for mean plaque per papillum, except that urine pH also becomes an independent factor ($P = 0.001$, adjusted multiple $R^2 = 0.606$).

*Conclusion*. Utilizing advanced digital video and endoscopic equipment, we have achieved the most accurate estimation of papillary plaque coverage to date. Our findings support the idea that urine volume and calcium are the main correlates of plaque coverage.

**Key words:** urine volume, urine calcium, Randall's plaque, renal papilla, urolithiasis.

Received for publication April 1, 2003
and in revised form June 4, 2003
Accepted for publication July 29, 2003



During an extensive hand lens study of 1154 postmortem kidneys, Alexander Randall first observed cream-colored deposits beneath the urothelium of the renal papillum, near its tip [1]. He found the deposits, which he called plaques, were interstitial, did not involve the tubule lumens, and seemed to be calcium [2, 3]. He hypothesized that plaque, once exposed to urine by erosion of overlying urothelium, would be an anchored nidus for calcium oxalate overgrowths. We have extended these observations using intraoperative papillary biopsies and have shown that plaque originates in the basement membranes of the thin loops of Henle and are entirely apatite [4]. Among calcium oxalate stone formers, and normal subjects, plaque is of the same histologic character, but the stone patients have far more extensive deposits. Of interest, four patients with stones from intestinal bypass for obesity had no interstitial deposits at all, but, rather, intratubular deposits, which were also apatite, even though all formed stones were calcium oxalate.

Given their apparent origins in structures closely related to water conservation, and tubule fluid concentration, we have hypothesized that plaque, if accurately quantified, would increase directly with urine calcium excretion, and inversely with urine volume. High calcium movement through tubules, as occurs in hypercalciuria [5], if coupled with increased water extraction, could increase calcium concentrations along the nephron, and create conditions favorable to accumulation of calcium salts in the interstitial spaces that would, in turn, lead to calcium solid phase deposits. Recent studies by others [6] have shown that plaque as assessed by endoscopic inspection was more prevalent among stone formers than in those undergoing endoscopy for conditions other than stones. Past investigators [7] also showed that higher plaque severity appeared related to hypercalciuria, but a significant correlation was not demonstrated.

In order to test our hypothesis more exactly, we have devised methods to quantify plaque area during percutaneous nephrostolithotomy (PNL), using advanced

digital video and high-resolution endoscopic imaging equipment. In addition, 24-hour urine risk factors were measured, along with indices of crystallization inhibition, that included estimates of calcium oxalate crystal growth inhibition [8] and measurements of the upper limits of metastability [8], both thought to reflect endogenous defenses against crystallization. Finally, we applied these methods to exactly those patients and nonstone-forming subjects whose renal biopsies we have detailed elsewhere [4], so the histologic nature of the plaque is entirely interstitial, without tubule lumen involvement.

## METHODS

### Patients and clinical measurement

A total of 18 patients (ten males and eight females with mean age of 51.3 years) were enrolled in this Institutional Review Board–approved study. The cohort included 14 idiopathic calcium stone formers and four control patients with no prior clinical or family history of nephrolithiasis. The calcium oxalate patients underwent PNL for treatment of their large stone burdens. Percutaneous access was obtained with a fluoroscopic triangulation technique. A Nephromax balloon (Boston Scientific, Natick, MA, USA) was used for tract dilation in all cases, along with a 30 F access sheath. The control population consisted of patients with diagnoses of renal cell carcinoma and no prior history of nephrolithiasis who underwent laparoscopic radical nephrectomies for treatment of their primary disease. A subcostal transperitoneal port configuration was utilized. These kidneys underwent endoscopic mapping following specimen extraction.

At least 1 month after surgery, all 14 stone patients collected two 24-hour urines on their self chosen diets. The four control patients collected their urines prior to nephrectomy. All medications that could alter calcium metabolism were discontinued at least 2 weeks prior to the collection, including thiazides, allopurinol, and potassium or sodium citrate salts. Samples were collected as has been described by this laboratory [9]. Measurements included volume, calcium, oxalate, citrate, pH, sodium, potassium, chloride, sulfate, ammonia, creatinine, and uric acid. In addition, an aliquot of urine was dialyzed and assayed for its ability to slow the growth of calcium oxalate seed nuclei, in vitro [8]. The whole urine, within 1 day of collection, was assayed to determine the upper limits of metastability for calcium oxalate and calcium phosphate (as brushite, calcium monohydrogen phosphate), using the protocols we have described elsewhere[8].

### Endoscopic mapping protocol

All patients underwent a retrograde pyelogram to delineate the collecting system anatomy in the kidney of interest. In the stone patients, collecting systems were opacified via a ureteral catheter placed to facilitate percutaneous access. For the control patients, a ureteral catheter was inserted following removal of the kidney.

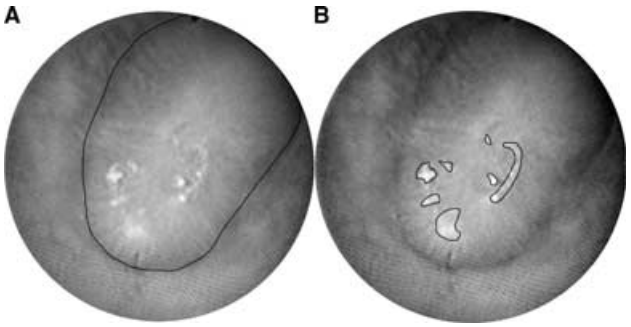


**Fig. 1. Digitized image of papillary plaque.** The papillary border was outlined (*A*) in order to measure the total number of pixels encompassed within the papillary domain; after this, the individual plaque borders were outlined (*B*) so that the total pixel number could be measured within the plaque area domain.

For the PNL cases, all accessible papilla were sequentially visualized, or mapped, through the established access tract after completion of stone removal. Papilla immediately adjacent to the calyx of access were visualized with a 27 F offset rigid nephroscope. More peripheral papilla were examined with 15 F analogue fiberoptic flexible nephroscopes. An identical process was employed for the nephrectomy specimens following the creation of a small pyelotomy; however, we used a newly available 15 F digital flexible nephroscope (Pentax, Orangeburg, NY, USA) in these kidneys. Video footage of the endoscopic mapping procedure was recorded on either S-VHS or DV tape format. Each patient's footage was then reviewed using the retrograde pyelogram as a reference to confirm the location of each papillum. The footage was edited and converted to the moving pictures expert group (MPEG) 1 standard for video compression and decompression to create digital movies providing real-time papillary views. Representative color and black and white still images of each papillum were also captured from the final edited footage.

A single investigator (J.E.L.) reviewed the MPEG-1 videos and still images to identify the location, extent, and distribution of any papillary plaques. After all plaque and papillary borders were outlined on prints of the still images, the borders were recreated on digitized versions of the images using Adobe Photoshop (Adobe Systems, San Jose, CA, USA) software (Fig. 1). The histogram function was then utilized to determine the total number of pixels that comprised the plaque areas and the papillary surface area. For each papillum, the fraction of papillary surface area involved with plaque was then determined by calculating the ratio of total plaque pixels to total papillary pixels.

### Statistical analysis

The fraction of papillary surface area covered by plaque was calculated for the upper pole, interpolar, and

lower pole regions by adding together all individual values for separate papilla. The fractional values of the three poles were then added to create a single figure representing the total fraction of surface area involved with plaque and divided by three to achieve a mean for the polar regions. In a second calculation, we added together the fractional coverage for each of the individual papilla and divided by the total number of papilla in order to obtain the mean fractional plaque coverage per papillum. The distributions of values for total surface percent were highly skewed to the left (low values). After log (10) transformation, the distributions were more normal in character. The relationship of log-transformed surface coverage to urine measurements was assessed using simple regression and general multivariate linear modeling (Systat Software, Inc., Richmond, CA, USA), in which the fractional plaque coverage data were dependent, and all urine measurements were entered as independent, with stepwise forward analysis. We analyzed separately mean coverage per pole and per papillum.

## RESULTS

Mean plaque coverage by pole differed between stone formers and nonstone formers (7.4% vs. 0.5%, $P = 0.012$), as did coverage per papillum (7.6% vs. 0.6%, $P = 0.011$). Log transformation did not affect the significance of the differences for either index of plaque coverage. Using simple correlations, and considering stone formers and nonstone formers together, plaque coverage per papillum was correlated significantly with urine volume and calcium excretion ($P = 0.004$ and 0.029, respectively). The same was true for plaque coverage per papillum ($P = 0.004$ and 0.043, for urine volume and calcium excretion, respectively). None of the other urine measurements had any significant correlations. We deliberately did not correct for multiple correlation, because we wished to see all those possibly present.

Considering mean plaque coverage per pole, using general linear models, urine volume and calcium excretion each had an independent correlation ($-1.004$ and 0.002, urine volume and calcium excretion, respectively, $P = 0.001$ and 0.03, respectively). Considering mean plaque coverage per papillum, we found much the same, except that urine pH also had an independent effect ($-0.54$, 0.003, and $-0.467$ partial correlation coefficients, $P = 0.015, 0.003$, and 0.031, respectively, for urine volume, calcium excretion and pH). In this model, the independent correlations were 0.566, $-0.431$, and $-0.378$ for urine calcium, volume, and pH, respectively, with $P$ values given above. Overall, urine volume and calcium excretion are the main correlates of plaque coverage, whichever way we calculate it. Higher calcium and lower volume increase coverage, and conversely. Urine pH correlates inversely with plaque coverage, for unknown reasons, but only when considering coverage per papillum.

Urine volume was the most striking of the three significant partial correlations we found for plaque coverage per papillum (Fig. 2, upper left panel). Urine calcium (Fig. 2, upper middle panel) showed a broad and scattered relationship. Urine pH (Fig. 2, upper right panel) showed a somewhat more impressive relationship that was inverse. A score using all three variables (Fig. 2, lower left panel) was highly correlative with plaque coverage (adjusted squared multiple $R = 0.606$, $F = 9.705$, $P = 0.001$), as was the score using only urine volume and calcium excretion (Fig. 2, lower right panel; adjusted square multiple $R = 0.48$, $F = 8.85$, $P = 0.003$).

In a similar manner, we found that for mean percent coverage per pole, urine volume was most striking (results not shown). A score using urine volume and calcium with the partial correlation coefficients derived from our general linear model shows a strong positive correlation with the average percent coverage per pole (adjusted squared multiple $R = 0.521$, $F = 10.24$, $P = 0.002$ for the regression). Overall, urine calcium and volume account for a very considerable amount of the variation in plaque coverage per pole.

These results strongly support the hypothesis that urine volume and calcium excretion, with a contribution from urine pH when fractional plaque coverage per papillum was considered, account for much of the difference in plaque coverage between stone formers and nonstone formers. As an additional approach to quantify a test of this hypothesis, we constructed analysis of variance (ANOVA) models for plaque coverage per pole and per papillum in which urine calcium excretion and volume were entered as covariates, and stone formers vs. nonstone formers was the factor. For coverage per pole, adjusted least squared means no longer differed (0.361 vs. 0.167, $P = 0.67$, stone formers vs. nonstone formers, respectively). Matters were similar for coverage per papillum (0.398 vs. 0.174, $P = 0.625$, stone formers vs. nonstone formers, respectively). This is not to say that having stone disease does not cause plaque via mechanisms beyond those involving urine volume and calcium excretion, but it does support the idea that this simplicity of mechanism is not unfeasible.

## DISCUSSION

Alexander Randall developed a significant hypothesis about the development of calcium nephrolithiasis based on his detailed examination of autopsy kidneys, in which he noted the presence of interstitial papillary deposits which he referred to as plaque [2]. When these deposits become exposed to the urinary space following erosion of the overlying urothelium, Randall thought that they would become nidi for the formation of calcium stones. Despite the revolutionary nature of these observations, Randall's original studies have been criticized in that they were performed in a postmortem series, with no means

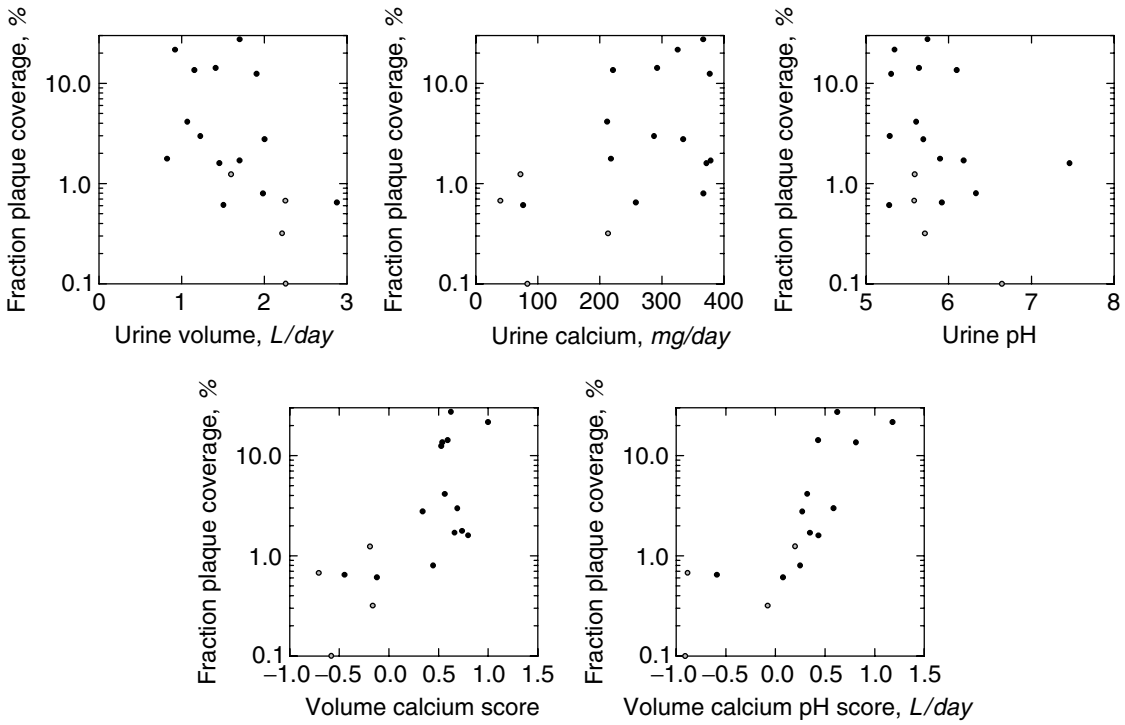


**Fig. 2. Urine correlates of papillary plaque.** Fractional plaque coverage per papillum varies inversely with urine volume (upper left panel) among stone formers (•) and nonstone forming control subjects (∘). Plaque coverage varies with urine calcium excretion (upper middle panel) and is inverse to urine pH (upper right panel). A composite multivariate regression score using urine volume and calcium excretion (lower left panel) and one that includes urine pH as well (lower right panel) strongly correlate with plaque coverage.

of confirming past histories of nephrolithiasis. Therefore, follow-up studies in a population of well-documented stone formers would be required to substantiate Randall's hypothesis. Until recently, however, further insight into the correlations between Randall's plaques and the stone-forming population has been limited.

Low and Stoller [6] were the first investigators to characterize the appearance and prevalence of Randall's plaques in patients undergoing endoscopic treatment for nephrolithiasis. Their initial study revealed that plaque was more prevalent in stone formers as opposed to those undergoing endoscopy for conditions unrelated to stone disease. In particular, those with a history of calcium stones were found to have significantly more plaque. In a follow up study, the results of 24-hour urine metabolic studies were correlated to endoscopic findings [7]. The authors concluded that patients with hypercalciuria trended toward increasing plaque severity; however, no significant relationship was demonstrated. These studies highlight a possible association between calcium nephrolithiasis, especially in the setting of hypercalciuria, and Randall's plaque.

Our study has attempted to build upon the foundation established by Low and Stoller [6] by creating a method to accurately estimate the amount of papillary plaque present in both stone-forming and nonstone-forming patients. To achieve this goal, we utilized digital video technology in conjunction with the most advanced flexible endoscopy equipment available to assess papillary surfaces during nephroscopy. A combination of digital real-time footage of endoscopic papillary views, and representative digital still shot prints of each papilla, were used to identify plaques. Image editing software was then used to outline the identified plaques in order to provide the fractional plaque coverage estimates used here.

The refinements incorporated into our papillary mapping protocol allowed us to achieve the most accurate plaque surface area estimates to date, as previous studies documented only the presence or absence of plaque [6], or succeeded in characterizing only broad ranges of plaque severity (mild, intermediate, severe) [7]. Our study is the first to show that plaque coverage fraction as we measure it correlates strongly with urine measurements made at an entirely different point in time, thus confirming that the urinary milieu has important pathophysiologic significance in the case of calcium nephrolithiaisis.

The present results lend support to the idea that interstitial plaque deposits arise from driving forces that are reflected in urine calcium excretion and urine volume. Plaque coverage, as expressed as a percentage of total papillary area, varies inversely with urine volume, and directly with urine calcium excretion, and the two variables have independent contributions to the regression as analyzed using standard general linear modeling. Because we have used patients from whom we have obtained

biopsies, we can be certain that the plaque whose surface we measured is indeed of the interstitial character that Randall described [2, 3] and spares tubule lumens. Of the two variables, urine volume is the more robust, as judged from the significance value for its coefficient. This would strongly support a physiologic link between water extraction in the renal medulla and papilla and plaque formation, that is, in turn, consistent with the apparent histologic origins of plaque in the basement membranes of the thin segments of the loops of Henle [4].

Our measurements reflect conditions in the renal tubules, yet plaque is entirely interstitial; how can these be reconciled in a form that leads to reasonable hypotheses of plaque formation? The most immediately available hypothesis is that when water conservation is increased, tubule fluid in the thin limbs of Henle's loop becomes maximally concentrated. This fluid is known to have high concentrations of calcium and phosphorus, as well as a pH near that of blood [10]. Although epithelial permeabilities are low for calcium and phosphorus [10], time is not a limiting variable because tubule fluid is always present. What matters is the net resultant of ion movement into the interstitium opposed by removal by the vas recta. Because the latter tend to accumulate materials in the papilla via countercurrent exchange [11], interstitial concentration in the vicinity of the thin limbs could rise proportional to water conservation. Idiopathic hypercalciuria, the usual cause of high calcium excretion in stone formers [5], is known to arise via increased vitamin D activity [12]. In particular, intestinal calcium absorption is elevated. After eating, one would predict pulses of increased filtered load of calcium that deliver high calcium fluxes into the thin limbs. The same is true, potentially, for phosphorus.

Alternative hypotheses are easily constructed. Plaque coverage per papillum varies inversely with urine pH. The lower the urine pH, the higher the delivery of bicarbonate into the deep medulla by collecting duct proton-secreting cells. Such a mechanism would raise interstitial pH and foster plaque that is composed of calcium phosphate. Estimates of crystal inhibition, although abnormal in stone formers [8] of both genders, do not bear any apparent relationship to plaque. Likewise, upper limits of metastability, that also are abnormal in stone formers [8], bore no relationship to plaque coverage.

Altogether, and allowing for our uncertainties about pathophysiologic linkages, this study establishes that plaque formation cannot be easily disentangled from mechanisms that control calcium excretion and water conservation. In fact, plaque could easily arise from as simple a cause as concentration of calcium salts in the thin limbs of the loop. Our work gives rise to natural and testable hypotheses but is of course unable to go beyond the present available data.

The superior resolution afforded by our digital endoscopic equipment also provides the opportunity to examine specific plaque distributions and characteristics in various stone-forming populations. With further study, we speculate that it may be possible to link specific plaque appearances to particular urine metabolic abnormalities.

## CONCLUSION

We have utilized advanced digital video and endoscopic equipment in combination with image editing software to achieve the most accurate estimation of papillary plaque coverage in both idiopathic calcium stone formers and nonstone formers to date. When correlated to 24-hour urine data, we have found that two primary factors, urine volume and calcium excretion, are involved in the formation of interstitial plaque. Examination of specific plaque patterns and characteristics, with correlates to urine metabolic findings, may be possible with this technique.

## ACKNOWLEDGMENTS

The authors wish to acknowledge the assistance of William A. Geary, M.D., Ph.D., Jeffrey A. Moody, M.D., and Philip Blomgren with this project. This work was supported by grants from NIH P01 DK56788 and the American Foundation for Urologic Disease.

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